THE CONTROL OF THE DERMAL MELANOPHORES IN ELASMOBRANCH FISHES

GEORGE H. PARKER AND HELEN PORTER

(From the Woods Hole Oceanographic Institution [1] *and, Marine Biological Laboratory, Woods Hole, Mass.)*

Introduction

The color changes in the elasmobranch fishes have received scant attention. Aside from the negative results recorded by Schaefer (1921) for *Raja clavata* and *Raja batis,* the only account of this subject known to us is that given by Lundstrom and Bard (1932) in a paper devoted to the action of the pituitary secretions on the color changes in the dogfish, *Mustelus canis.* In this paper it is shown in a very conclusive and adequate way that hypophysectomy is followed by a permanent paling of the fish and that this paling can be changed into a temporary darkening by the injection of pituitary extract. It is further shown by these authors that the changes thus experimentally induced agree in general with those normally excited in this fish by alterations in the tint of the background.

In their account of these changes Lundstrom and Bard give a very full description of the alterations of the fish from light to dark, but the reverse change is passed over with very slight comment. Although no specific statement is made by them on this point, it seems probable from the contexts in their paper that they regard the light phase of the dogfish as due to a decrease in the amount of the pituitary secretions in the fish's blood or possibly to a complete absence of these secretions from this fluid. According to them, this condition is in contrast to that in which there is an abundance of these secretions which thus induces the dark phase in the fish. That this dark phase is due to pituitary activity seems to us adequately and sufficiently shown and in this respect our own results fully agree with those of Lundstrom and Bard, but that the light phase is the consequence of the mere absence of the pituitary secretions appears to us quite unlikely as the observations recorded on the following pages tend to prove. Our own work was carried out on the smooth dogfish, *Mustelus canis,* partly at the Woods Hole Oceanographic Institution and partly at the Marine Biological Laboratory. To the Directors of these two establishments we wish to express our sincere thanks for the facilities and materials supplied us by which our investigations were made possible.

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Changes in the General Coloration of the Dogfish

When a light-colored dogfish is placed in a black-walled tank illuminated from above, the fish quickly becomes dark. The time required for this change is from half an hour to an hour or two, and the subsequent retention of the fish in the dark-walled aquarium does not deepen further its tint.

Whether of light or dark shade in the beginning, dogfishes from which the eyes have been removed become quickly dark and remain indefinitely so irrespective of the shade of their surroundings. Such blinded fishes attain as a rule a deeper gray than the maximum shown by the normal fishes in a dark-walled tank. In fact, the darkest dogfish that we have ever seen was one whose eyes had been removed.

Dark dogfishes placed in an illuminated white-walled tank become gradually very light. Occasionally under such circumstances they fail to reach the extreme of light coloration and will maintain even in fully light surroundings a gray tint. This failure of full response has also been noticed by Miss Lundstrom, who called our attention to the frequent occurrence of cataract-like obscurities in the dioptric portion of the dogfish eye and suggested these defects as a possible explanation of this failure on the part of certain dogfishes to turn fully light. Unfortunately we were unable to test this suggestion. The majority of dogfishes, however, become extremely light in light surroundings and remain so. This paling is often associated with a pink tone that gives to the fish the appearance of a delicate blush. Such a coloration is doubtless brought about by the subdermal blood-vessels, which in consequence of the translucency of the skin in the light state show through this covering and thus affect the general tint of the fish.

The establishment of the light phase in the dogfish is a relatively slow process. A dark fish when put in a white-walled tank grows light at first rapidly and then very much more slowly till the maximum lightness is reached. This maximum is attained only after two or more days whereupon the fish remains indefinitely pinkish white.

What in general is thus slowly accomplished in a white-walled tank may be reached quickly by a subcutaneous injection of adrenalin chloride (Parke, Davis and Company). About five minutes after a dogfish has received a hypodermic injection of 0.5 cc. of diluted adrenalin, 1 part in 10,000, a general paling of the skin can be seen and half an hour later the fish is fully light. The degree of paling, even when 1 cc. of adrenalin is introduced into the fish, is seldom as pronounced as that seen in fishes from the white-walled tank, but occasionally the injected fishes reach what in our experience is the maximum of light coloration.

It is comparatively easy to show by placing the pectoral fin of a dog-

fish under the compound microscope that the dark phase of the fish is due to the expansion of the dermal melanophores and the light one to their contraction. Excellent figures of these conditions have already been published by Lundstrom and Bard (1932).

Fin Bands

If in a moderately dark dogfish deep cuts transverse to the rays of any fin are made or similar cuts are inflicted on the body, these cuts give rise to light bands or splotches which are clearly visible in the skin for many hours or even days thereafter. If the spinal cord of a dogfish is transected in the posterior part of the body, the portion of the fish behind the cut becomes irregularly slightly lighter. The light bands are best seen when they are produced on the pectoral fins and are most easily induced by making a well circumscribed cut completely through the fin from one face to the other at right angles to the rays. Such an incision is conveniently made by means of a chisel with a cutting edge of about one centimeter.

In the pectoral fin the course of the main blood-vessels can be easily seen through the translucent substance of that organ and the cut may be made either distal or proximal to the chief vessels, thus leaving the blood supply to the fin essentially undisturbed. The cut necessarily severs a number of the smaller vessels, but, if its position is well chosen, it can be shown to introduce no serious interference with the circulation. After such a cut has been made a light band quickly develops extending from the cut to the free edge of the fin. This band assumes the width of the cut, never more, and always extends over the fin distally from the cut, never proximally. It follows very closely the lines of the fin rays. Its detailed nature and fate depend upon the light or dark condition of the dogfish in which they are induced.

In a dark dogfish the band is a lightish area of irregular outline (Fig. 1). It is seen with certainty in from ten to fifteen minutes after the cut has been made and it reaches its maximum in about a day. After this it gradually fades out, to disappear completely in from two to three days. A narrow band three to four millimeters wide will, however, disappear in a little over a day; one of a centimeter wide will remain visible for as much as two to three days. The light band fades by being replaced with the dark coloration of the surrounding fin area, the center of the band near the periphery of the fin being the last to disappear (Fig. 2).

When a cut is made in the pectoral fin of an eyeless dogfish, a light band also appears in about a quarter of an hour and then follows the usual course of disappearance.

In a light dogfish the band is much more clearly defined in outline

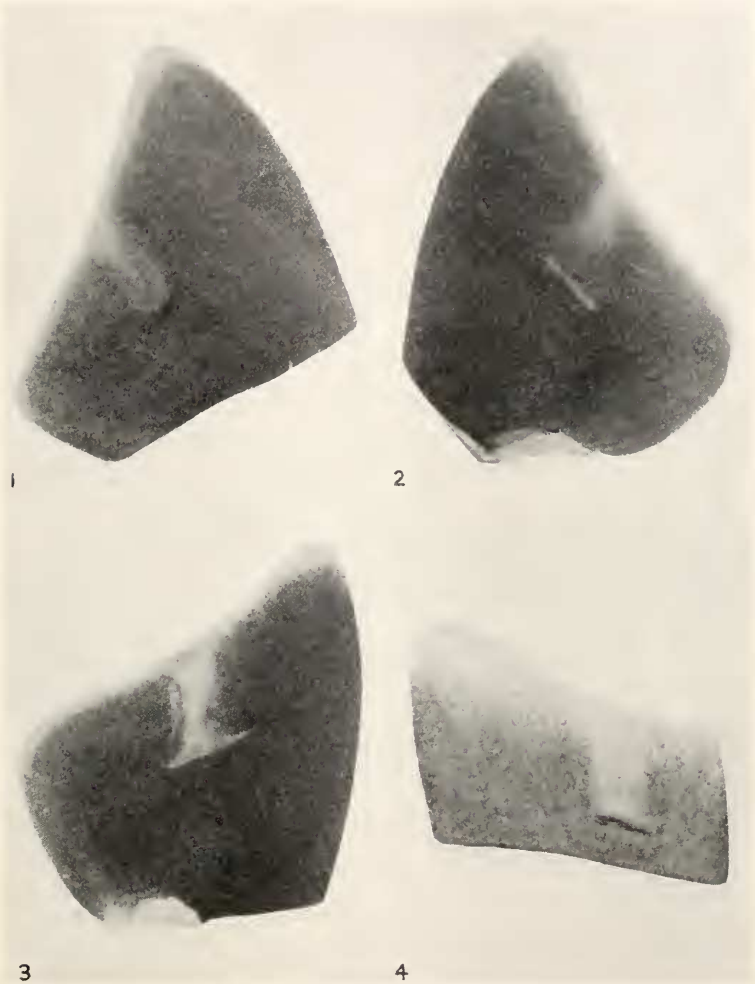

PLATE I

Dorsal View of Pectoral Fins from the Dogfish, Mustelus canis

FIG. 1. A fin from a dark dogfish, showing a light band which appeared as a result of an incision transverse to the direction of the fin rays and about a centimeter and a half from the edge of the fin. The figure represents the state of the band about an hour after the cut had been made.

FIG. 2. A fin from a dark dogfish, showing a light band in process of gradual disappearance. The band was produced by the same kind of a cut as that shown in Fig. 1, but in the course of two days it became partly obliterated by the invasion of the general dark coloration.

FIG. 3. A fin from a dark dogfish, showing a light band in process of disappearance, an operation here locally checked by a longitudinal cut on one side of the band.

FIG. 4. A fin from a light dogfish, showing a light band several days after the initial transverse cut had been made.

than in a dark one. Its edges are sharply marked and they can be traced from the ends of the cut to the edge of the fin (Fig. 4). Although the fish may be extremely light in color, the band is always still lighter and agrees in tint with the white border of the fin. Unlike the light band on the dark fish, that on the light fish seems never to be obliterated. At least in all light dogfish kept by us in the laboratory tank the light band has persisted as long as the fish has lived, a period of at most about five days. When from a light dogfish with a light band the eyes are removed the fish darkens and the band eventually disappears as it does in an ordinary dark fish in which it had been induced by a cut.

Discussion

We are entirely in accord with Lundstrom and Bard in holding the opinion that the dark phase of the dogfish is due to the action of an expanding hormone carried to the melanophores from the pituitary body by the blood. We are convinced of the correctness of this opinion partly because of the facts advanced by Lundstrom and Bard and partly because of our own results. If the blood of a dark dogfish is drawn, defibrinated, and injected subdermally into light and into dark dogfishes, it will be found to have no effect upon the skin of the dark fishes but to produce a well-defined dark area on that of the light ones. This dark area, which may measure several centimeters in diameter, surrounds the point of injection and when examined under the microscope can be shown to have been produced by the expansion of melanophores.

Another observation that supports the general conclusion under consideration was made on operated fishes. If in a light dogfish a longitudinal cut parallel with the fin rays of the pectoral fin is made near the edge of a light band and the dogfish is put in a dark-walled tank, as the light band disappears it will last be seen next the longitudinal cut and on that side of it adjacent to the axis of the original band (Fig. 3). This method of disappearance is consistent with the idea of an invading, fluid-borne, expanding hormone such as has been claimed by Lundstrom and Bard.

For these and other reasons we are fully convinced that the dark phase of the dogfish results from the application of a blood-soluble, expanding hormone to the dermal melanophores.

The light-colored bands and their reactions as we have described them seem to us to be equally conclusive evidence that the light phase of the dogfish is dependent upon nervous action. To be sure, these bands follow the courses of the smaller blood-vessels and might therefore be attributed to an operative disturbance in the circulation. But, as already stated, the cut can be made either proximal or distal to the chief

blood-vessels of the fin and the resulting band, which may easily be two or more centimeters long, is in large part so far from the exciting incision that the local circulation is completely undisturbed. This can be easily demonstrated by inspecting under a compound microscope the living pectoral fin of an operated dogfish. If the light ventral face of such a fin be examined, the flow of blood in the capillaries within the limits of the band can be seen to be as free and as abundant as it is in the regions outside the band. There is thus no reason to attribute the formation of the band to any circulatory irregularity, and the conclusion that is naturally arrived at is that the only other radial system, namely the nerves, is responsible for the bands.

The fin nerves run closely parallel to the fin rays and much more accurately so than the blood-vessels do. Since in light dogfishes the edges of the light bands follow very closely the courses of the rays, they must also follow the lines of the nerves. Hence we believe the light bands to be dependent upon the nerves. When a transverse cut is made in a fin a number of fin rays with their attendant nerves are severed and this severance is so vigorous a stimulus for these nerves that they excite the melanophores of their region to full contraction. The result of this response is that a light band is produced corresponding in extent to the area of distribution of the contracting nerves. Such a view coincides completely with what we know of the distribution of light spots and bands that result from skin cuts on other parts of the body of the dogfish. Spots or bands thus induced always extend from the region of the cut in the distal direction of the nerves and in no other. They are due in our opinion to the excessive activity of the severed contracting nerve fibers.

How long their contracting activity can be kept up we do not know. The bands in a light dogfish will maintain themselves essentially undiminished for as much as five days, the longest period that we have been able to keep an operated dogfish alive in the experimental tank. This period is much too short, judging from our results on *Fundulus* (Parker and Porter, 1933), to reach a condition of nerve quiescence and in our opinion the fin nerves concerned with melanophore contraction in the dogfish must be regarded as in more or less continuous action as a result of the irritation of the wound for a period of at least five days.

During this period, however, there appears to be in the dogfish a certain amount of decline in activity. This is seen in the disappearance of the light bands in dark dogfishes. As already pointed out, the light bands which are indefinitely persistent in light fishes disappear in at most two or three days in dark fishes. This disappearance we believe to be due to the action on the contracted melanophores of the expanding,

blood-borne hormone which counteracts the contracting action of the nerves. When the fin nerves are first cut in a dark dogfish they are so vigorously stimulated that their activity predominates locally over that of the expanding pituitary hormone and the light band is formed. As time goes on, however, the melanophores of the band come more and more under the influence of the expanding hormone, a step rendered possible, we believe, by the subsidence in activity of the contracting nerve fibers. Thus the disappearance of the light band from the fin of a dark dogfish depends, in our opinion, in part at least on the gradual decline in the activity of the contracting fibers whereby the expanding hormone may assert itself. As already stated, we have never seen instances among dogfishes where this decline was complete, for if a dark dogfish in which the band has disappeared is put in a white-walled tank the band will reappear as the fish lightens. Nevertheless, we believe that in time this activity might completely subside, as it has been shown to do in the expanding fibers of *Fundulus* (Parker and Porter, 1933).

It must be clear from what has been stated that in our opinion the darkening of the dogfish skin is due to the action of an expanding blood-soluble hormone on the dermal melanophores of the animal. It must also be clear that the paling of the dogfish skin results from the action of contracting nerve fibers on the melanophores, an action which when excessive may overcome that of the expanding mechanism. Do the contracting nerve-fibers produce a contracting substance or neurohumor comparable to the pituitary expanding hormone? Thus far we must answer this question in the negative and in consequence of two lines of evidence. First, when the defibrinated blood of a light dogfish is injected into a dark one, the skin of the dark individual does not lighten, showing that such blood carries no dissolved contracting neurohumor such as is stated by Meyer (1931) to occur in certain flatfishes. Secondly, if the width of a newly-produced band in the pectoral fin of a light dogfish is accurately measured and if this measurement is compared with a second one of the same band in the same place made three days later, no increase in width can be noticed. This result is opposed to the view that the light band is due to a water-soluble neurohumor produced by the contracting nerve fibers and more or less like the expanding blood-borne hormone. If such a substance were present it most certainly would induce an expansion of the band. The evidence here presented, however, does not preclude the possibility of a locally produced contracting neurohumor insoluble in blood or lymph, but it makes clear that if such a neurohumor is present, it is probably lipoid-soluble and would therefore be essentially local in action.

The general conclusion to be drawn from this discussion is that the

color changes in the dogfish are of two types, one from light to dark due to an expanding, pituitary hormone carried from the gland to the melanophores by means of the blood and other body fluids, and another from dark to light due to a contracting local action of nerves. It is interesting to note that of the many animals in which the cutting of nerves produces a change in coloration, the dogfish is the first one to be recorded in which this change is a paling. All other forms exhibit a darkening when their chromatophoral nerves are severed. This we think is due to the fact that in the dogfish, unlike other fishes, probably only contracting nerves are present and that consequently in this fish these nerves have no others with which to compete as the contracting nerves of other fishes apparently have. On stimulation these nerves in the dogfish therefore assert themselves without hindrance. The dogfish is further peculiar in that it presents a melanophore system in which the expanding action is unihormonal, modeled after that of an amphibian, and the contracting action nervous like that in most other fishes.

Summary

1. The common dogfish, *Mustelus canis*, has a dark phase and a light one due to the expansion and the contraction of its dermal melanophores.

2. The dark phase is induced by pituitary secretions carried from the pituitary gland to the melanophores by the blood and lymph as described by Lundstrom and Bard.

3. The light phase is induced through the action of contracting nerves, and is not merely the result of the absence of pituitary secretions.

4. The expanding hormone is water-soluble. The contracting one, if there be such, is apparently not water-soluble.

5. The dogfish is remarkable as the first form to be described in which the cutting of nerves induces a contraction of the melanophores and a consequent lightening of the skin.

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